Accumulation and fractionation of trace metals in a Tunisian calcareous soil amended with farmyard manure and municipal solid waste compost

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Abstract

A field plots experiment was carried out to assess the effects of repeated application of municipal solid waste compost in comparison to farmyard manure on the accumulation and distribution of trace metals, as well as organic carbon and nitrogen in Tunisian calcareous soil.

Compared with untreated soil, the application of the two organic amendments significantly increased the organic carbon and nitrogen contents of the soil. Particle-size fractionations showed that carbon and nitrogen were mainly found to occur in the macro-organic matter fraction (80%). The two organic amendments significantly increased organic carbon in the macro-organic and mineral >150 μm fraction and the 150-50 μm fraction, as well as the organic nitrogen in 150-50 μm and macro-organic fraction. Compared with farmyard manure, municipal solid waste compost significantly increased total Cd, Cu, Pb and Zn contents in the topsoil. These trace metals were mainly present in the macro-organic matter fraction. Significant increases of Cu, Zn and Pb were detected in the 150-50 μm, <50 μm and macro-organic fractions after application of municipal solid waste compost. A significant increase of Cd content was only observed in the 150-50 μm fraction. The trace metals also showed different fractionation patterns when the BCR sequential extraction scheme was applied on untreated and compost-treated soil. The residual fraction was found to be the major fraction, especially for Cu, Cr, Ni and Zn. In contrast, Cd was mainly present in the acid-extractable and reducible fraction, whereas Pb was mainly associated with the reducible fraction.

Keywords: municipal solid waste compost, organic carbon, nitrogen, trace metals, BCR sequential extraction, particle-size fractionation

1. Introduction

In the Mediterranean region, difficult climate conditions and inadequate land management have led to a reduction in the organic matter content of soils [1]. This effect is intensified by the limited availability and high cost of manure and the popular use of mineral fertilizers. However, maintenance of adequate organic matter levels in the soils is very necessary to

maintain soil fertility and sustainable crop production. Therefore, new sources of organic matter have been tapped, such as municipal solid waste compost and sewage sludge [2,5].

In recent decades, the volume of municipal solid waste increased in Tunisia. Composting these urban residues contributes to reducing the amount of waste, which makes its management less difficult. Moreover, the produced low-cost compost could be used as amendment in agriculture, meanwhile recycling its valuable components: organic matter, N, P and other plant nutrients [2,6]. Application of the municipal solid waste compost to agricultural soils was previously found to improve micronutrient complex formation, which increases availability of micronutrients to plants, to stimulate the presence of beneficial soil organisms and to reduce the presence of plant pathogens. Moreover, it seems to increase water holding capacity, soil buffering and cation exchange capacity and it improves soil porosity [4, 7-9]. The quality of such municipal solid waste compost was found to depend on many factors, including the design of the composting process, feedstock source and proportions used, composting procedure and length of maturation [9].

Despite the beneficial effects, high metal concentrations were observed in the compost due to the use of urban wastes. These limit its utilization in agricultural soils as excessive loads of contaminants could negatively affect soil fertility. Moreover, the presence of metals can constitute a long-term environmental hazard as metals have very long residence times in soils. In addition, transfer of toxic metals to the human food chain may occur since absorption by plant roots is one of the main routes of entrance of trace metals in the food chain [8,10,11].

Trace metals are mainly associated with solid soil components and exist in various physico-chemical forms. Total concentrations of trace metals in compost amended soils provide little or no indication of their specific bioavailability, mobility and reactivity [12]. Consequently, the knowledge of metal speciation and distribution between different size fractions in compost treated soils may be useful to quantify potential environmental effects. Therefore, sequential

extraction procedures were developed in order to predict retention, mobility and bioavailability of trace metals in soils, sediments and organic amendments [13-17]. A wide variety of extraction procedures have been used [18] and results obtained from applying chemical fractionation schemes are highly dependent on the extraction protocol used [19]. Besides the chemical speciation of trace metals in soils, size fractionation is used to predict the distribution of these elements between different particle-size fractions of the soils. Despite the great amount of work conducted on chemical speciation of trace metals in soils, little is known about the effects of applying municipal solid waste compost on the distribution of metals between different particle-size fractions in the soil.

The purpose of this study was to investigate the effect of seven successive years of municipal solid waste compost and farmyard manure application on trace metal fractionation and speciation in a Tunisian calcareous soil.

2. Materials and methods

2.1. Experimental Design

The field experiment was conducted in the experimental farm of the National Institute of Agronomy of Tunis (INAT) in the north of Tunisia. This site is characterized by a semi-arid climate. The total annual rainfall is approximately 500 mm, whereas the average air temperature is 18 °C. The lowest air temperature is 10.7 °C in January and the highest air temperature is 26.4 °C in July. The field experiment was designed as a randomized complete block trial with a total number of 24 plots. The dimension of each plot was 1.5 x 1.5 m^2. Plots were arranged in four repetitive blocks and were separated in all directions by a buffer zone of 1 m. The soil was loamy clay with low organic carbon content (Table 1) and was classified as

Xerofluvent. Six treatments were examined: (1) control without amendment (T); (2) 40 t ha^{-1} $year^{-1}$ of farmyard manure (M40); (3) 120 t ha^{-1} $year^{-1}$ of farmyard manure (M120); (4) 40 t ha^{-1} $year^{-1}$ of municipal solid waste compost (C40); (5) 80 t ha^{-1} of municipal solid waste compost (C80); (6) 120 t ha^{-1} $year^{-1}$ of municipal solid waste compost (C120). The amendments were applied each year from 1999 to 2005. They were applied to the soil surface by hand in September/October and incorporated to a depth of 10-15 cm by manual hoeing. Plots were manually weeded every month to avoid uptake of the amendments by plants. Initial characteristics and trace metal concentrations of the municipal solid waste compost, farmyard manure and soil used in this study are shown in Table 1.

2.2. Soil analysis

2.2.1. General soil properties

Soil samples were collected in 2006, one year after ending the addition of organic amendments by taking a composite of five cores using a hand auger (4 cm diameter) at a depth of 0-20 cm for each plot. The soil was homogenized, air dried, crushed, and passed through a 2-mm sieve for further analysis. The pH and electrical conductivity (EC) were measured on a 1:5 sample/water extract after shaking during 2 hours for pH and during 1 hour for EC [22]. Organic carbon was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate [20]. Total nitrogen was determined using the Kjeldhal method. Total trace metals concentrations were determined by aqua-regia extraction: 7.5 ml of concentrated hydrochloric acid and 2.5 ml of concentrated nitric acid were added successively to one gram of soil. This suspension was allowed to react overnight and subsequently heated during two hours. After cooling, the solution was filtered and diluted to 100 ml with nitric acid solution (2 M). The concentrations of trace metals were measured

using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Vista MPX, Varian, Palo Alto, CA, USA). For quality control, a certified reference sediment of the Scheldt estuary (CRM 277) was also analyzed in triplicate using the same method. Results varied from 92.5 to 101.7% of the certified aqua regia extractable values. Moreover, standards were always re-analyzed at the end of each batch of analyses. The analyses results were only accepted when the measured standard concentrations were within 95 to 105% of the certified value.

2.2.2. Particle-size fractionation

Fractionation of soil into different size fractions was performed by a method which was slightly modified from Mejboom et al. [21]. The separation was carried out by wet sieving of 50 g soil sample over three sequential sieves (250 µm, 150 µm and 50 µm) with deionised water. The soil was pushed through the sieves until the water passing the sieves became clear. The suspension passing the bottom sieve was collected and left to settle for about 48 h at room temperature. After settling, the clear supernatant was considered to contain only soluble compounds and removed, whereas the residue was considered as size fraction <50 µm. Size fractions 250-2000 µm and 150-250 µm were also collected, remixed, washed into buckets and swirled with a jet of water. The macro-organic material was separated from the mineral >150 µm fraction by decantation. Swirling and decanting were repeated until no floating organic matter appeared anymore. Consequently, four fractions (a macro-organic fraction and mineral >150 µm fraction, a 150-50 µm and a <50 µm fraction) were obtained. Suspensions were dried at 40°C for 48 h and stored for further analysis. Total metals were extracted from the fractions >150 µm, 150-50 µm and <50 µm by digestion with aqua regia and from the macro-organic matter by digestion with nitric acid after ashing the organic matter at 450-550°C for 2 h [22].

The concentrations of trace metals (Cd, Cu, Zn, Pb, Ni and Cr) were determined using ICP-OES (Vista MPX, Varian, Palo Alto, CA, USA).

Total carbon and nitrogen (TC and TN) in the whole soil, in the different soil fractions and in macro-organic matter fraction were analysed using a CNS analyser (Vario Max, Elementar, Germany).

2.2.3. BCR Sequential extraction

Different types of sequential extraction procedures have been developed and applied to fractionate trace metals in soils. We followed the standardized BCR sequential extraction method [16]. The developers of this method justified the use of a very strict standardized protocol as the only way to achieve comparability between data when using operationally defined procedures [23]. It distinguishes between only three fractions (acid-extractable, reducible and oxidizable) arguing that analysis of more fractions could increase error between fractions [24].

One gram of soil sample (<2 mm) was weighed into a 100 ml glass centrifuge tube. To extract the acid-extractable fraction (f_1) in the first step of the procedure, the soil sample was shaken with 40 ml 0.01 M CH_3COOH at a room temperature for 16h (overnight). To extract the reducible fraction (f_2) in the second step, the residue from the previous step was extracted by shaking the sample with 40 ml of 0.1 M $NH_2OH.HCl$ (adjusted to pH 2 with HNO_3) for 16 h at a room temperature. To extract the oxidizable fraction (f_3), 10 ml of H_2O_2 was added to the residue from the second step and the mixture was digested at room temperature for 1 h. It was then heated to near dryness in a water bath at 85°C. A second 10 ml of H_2O_2 was added and heated to near dryness again. After cooling, 50 ml of 1 M ammonium acetate was added to the moist residue and shaken for 16 h at room temperature. In a supplementary step, the

residual fraction *(fr)* was determined in the residue of the third step by digestion with 3:1 hydrochloric: nitric acid, heated to 150°C (aqua-regia).

To separate the extraction solution from the solid residue after shaking in each extraction step, the suspension was centrifuged at 3000g for 20 min. The supernatant was removed by filtration into a polyethylene container and analyzed immediately or stored in a refrigerator at about 4°C prior to analysis. Prior to each following step, the residue was washed with 20 ml of deionised water, followed by mechanical agitation for 15 min in an end-over-end shaker and removal of the supernatant after 20 min of centrifugation at 3000g. The concentrations of trace metals (Cd, Cu, Zn, Pb, Ni and Cr) were determined in each extract using ICP-OES.

2.2.4. Statistical analysis

Statistical analysis was performed using SPSS 10.0. Significance of effects was evaluated by one-way ANOVA. Duncan's test was used for comparison of means. Statistical significance of hypotheses was assumed when $p < 0.05$.

3. Results and discussion

3.1. Soil organic carbon, total nitrogen and trace metal contents

Soil organic carbon, total nitrogen and trace metal contents are presented in Table 2. Addition of both organic amendments during the seven successive years enhanced the total organic carbon and total nitrogen content for all rates. Organic carbon increased from 11 g kg^{-1} in the control treatment to 21 g kg^{-1} and 33 g kg^{-1} in the 40 and 120 t ha^{-1} farmyard manure treatments and to 21 g kg^{-1}, 29 g kg^{-1} and 35 g kg^{-1} in the 40, 80 and 120 t ha^{-1} municipal solid waste treatments, respectively. The increase of organic carbon content seems to be related the

application dose. Similar results were obtained by Celik et al. [7] who stated that the soil organic matter concentration at 0–15 cm depth in a clay loam soil was higher in plots amended with manure and compost during 5 years, compared to control plots and plots treated with mineral fertilizer.

N'Dayegamiye et al. [4] reported that the addition of municipal solid waste compost (20, 40 and 60 t ha^{-1}) increased the organic matter content of a sandy loamy soil. The total organic carbon content of two sandy Malian agricultural soils also increased after application of industrial Belgian compost [25]. Moreover, Weber et al. [26] showed that one year after the application of two kinds of commercial composts in a loamy sand soil, the soil organic carbon content increased significantly, but only when the composts were applied at medium (60 t ha^{-1}) and high rates (120 t ha^{-1}).

For soil total nitrogen, a positive effect of both organic amendments can also be observed. In fact, the addition of 40 and 120 t ha^{-1} of farmyard manure significantly increased the total nitrogen content from 1.1 g kg^{-1} in the control soil to 1.7 g kg^{-1} and 2.8 g kg^{-1}, respectively. After application of 40, 80 and 120 t ha^{-1} of municipal solid waste compost, the organic nitrogen content increased to 1.5 g kg^{-1}, 1.8 g kg^{-1} and 2.4 g kg^{-1}, respectively. Consequently, farmyard manure enhanced organic nitrogen in the soil insignificantly compared to municipal solid waste compost. This may be attributed to the organic nitrogen content being higher in manure compared to compost. Soumaré et al. [25] reported that application of industrial Belgian compost on two sandy Malian soils had variable effects on total nitrogen content. In the first soil, total nitrogen increased significantly when compost was applied at a rate of 50 t ha^{-1} while even lower doses (25 t ha^{-1}) showed a significant effect in the second soil. After one year of application of commercial compost on a loamy sandy soil, only the highest rate (120 t ha^{-1}) significantly increased total soil nitrogen [26].

Our study confirms the results of a previous experiment conducted at the same plot which revealed that the two organic amendments (manure and compost) applied during three successive years were a good supplier of organic matter [27]. Increasing the soil organic matter content improves the soil physical characteristics, such as soil water retention and movement, soil structure and porosity, and favours the establishment of carbon cycling [28]. Moreover, mineralization of organic matter in added organic amendments leads to an important release of nutrients for plants, in particular nitrogen. The kinetics of mineralization in amended soils depend on soil texture, moisture regime and the nature of added organic matter [29].

The application of municipal solid waste compost in order to ameliorate the soil fertility may however cause the introduction of various contaminants in the soils. The increase of soil trace metal concentrations is usually reported as one of the most common undesirable effects that may result from application of composts [25,26,30].

The application of farmyard manure at 40 and 120 t ha^{-1} during successive seven years had no significant effect on the trace metal contents in the 0-20 cm layer of our soil (Table 2). Only a slight insignificantly increase of Cu and Zn was observed after the addition the rate of 120 t ha^{-1}. Manures contain plant functional nutrient metals such as copper, zinc, manganese, iron, and may contain trace amounts of non-functional elements. These functional elements were due to the natural presence of micronutrients in feed as well as their use as dietary supplements [62]. This may be due to the low heavy metal concentrations in manure. Similar results were found by Qian et al. [63] who stated that three to five years of annual swine and cattle manure applications at low and high rates increase slightly the total and bioavailable copper and zinc in surface soil. Gil et al. [28] announced that after application of composted

cow manure to sandy clay loam soil in Spain, Cr, Ni, Pb and Cd concentrations in the soil were not significantly different from the initial values. Moreover, repeated applications of farmyard manure for 20 years at 10 t ha^{-1} y^{-1} did not result in a significant accumulation of trace metals in the surface layer of Fluvisoil [31].

The addition of Tunisian municipal solid waste compost at different rates however showed a significant increment of Cd, Pb, Cu and Zn at 0-20 cm depth in our soil, whereas Ni and Cr concentrations were not significantly affected (Table 2). Compared to the control soil, Cd contents only increased significantly after application at the highest rate of 120 t ha^{-1}) of compost. In contrast, Pb, Zn and Cu concentrations showed a significant increment already upon application of the lowest rate of 40 t ha^{-1}. The magnitude of increment seemed to depend on the application rate.

Similar results were reported by Canet et al. [32]. They found that application of three organic amendments during seven successive years to a clay-loam soil had different effects on metal contents of the 0-20 cm soil layer. Sludge and municipal solid waste compost significantly increased the trace metals contents in all cases, with the exception of Pb. This increment also corresponded to the load of metals supplied by the two organic wastes and therefore depended on the application rate. The use of ovine manure did not increase trace metal concentrations of the soil due to its low metal content. Weber et al. [26] announced that the application of commercial compost originating from a polluted region to a loamy sandy soil significantly increased the Zn, Pb and Cu concentrations at all rates (30, 60 and 120 t ha^{-1}). Contrary, Cr and Ni concentrations only increased significantly after compost supply at the highest rate (120 t ha^{-1}). Upon application of urban waste compost to a clay-loam calcareous soil, soil Cu, Zn and Pb contents were significantly greater after 6 years. The Cr concentration increased only in the last two years of application, whereas no significant differences were found in Ni and Cd concentrations between treated and untreated soil [33]. Results given by Walter et al.

[34] showed that after one and five years after cessation of biosolid application to agricultural soil during eight years, the total concentrations of trace metals were significantly higher compared to the control. This was observed for 50 and 100 t ha^{-1} rates, with the exception of Ni at low application rates. In sandy soil treated by three successive applications of municipal solid waste compost at a rate of 1.8 and 2.1 kg m^2, Madrid et al. [1] found an increase of trace metals contents in the 0-25 cm layer.

From the above, it should be clear that the addition of municipal solid waste compost to soil can enhance its trace metals contents. However, the magnitude of increment depends on the origin of the composts [8].

3.2. Relative abundance of the different particle size-fractions within the soil

The highest weight percentage of soil (80 %) was found in the size fraction <50 µm (Fig. 1). The other size fractions formed smaller amounts of soil weight. Application of municipal solid waste compost and farmyard manure had no clear effects on the particle size fractionation. These two types of amendments however significantly increased the percentage of macro-organic matter in the soil. This percentage varied from 1.35% in the control soil to 6.82% and 7.49% after addition of 120 t ha^{-1} of farmyard manure and municipal solid waste compost, respectively.

3.3. Organic carbon and nitrogen concentrations in the different particle size-fractions

The organic carbon (C) and organic nitrogen (N) contents in the different particle-size fractions are shown in Fig. 2. In the control soil, the largest C and N contents were found in the macro-organic matter fraction, followed by the size fraction <50 µm. In the soil amended

by 120 t ha^{-1} of municipal solid waste compost and farmyard manure, the largest C and N contents were observed in the macro-organic matter fraction, followed by the 50-150 µm fraction. The application of both organic amendments significantly increased the C content in all fractions, except in the <50 µm fraction where increments were not significant. In 50-150 µm fraction the C content increased from 8.8 g kg^{-1} fraction in the untreated soil to 20.1 g kg^{-1} fraction and 21.7 g kg^{-1} fraction in the plots treated with 120 t ha^{-1} of manure and compost, respectively. In the macro-organic matter fraction, the amount of C increased from 105 g kg^{-1} fraction in the control soil to 152.7 g kg^{-1} fraction and 152.3 g kg^{-1} fraction after application of farmyard manure and municipal solid waste compost, respectively.

The N content only increased significantly in the macro-organic matter fraction and the 50-150 µm fraction after the application of the organic amendments. The N content increased in 50-150 µm fraction from 0.8 g kg^{-1} fraction to 1.9 g kg^{-1} fraction and 2.2 g kg^{-1} fraction and in the macro-organic matter fraction from 3.4 g kg^{-1} fraction to 9.3 g kg^{-1} fraction and 10.4 g kg^{-1} fraction after the application of farmyard manure and municipal solid waste compost, respectively.

The results obtained in this study showed that the transfer of organic carbon and total nitrogen, added with the application of the two organic amendments, into the different size-fractions of soil starts from the coarser fractions towards the finest fractions. It presents a slow process and depends on the mineralization of the organic matter. Since the finest fractions (fine silt and clay) are not easily accessible, the transfer and accumulation of organic matter are only detectable after a long term for these fractions.

According to Accoe et al. [35], the largest content of soil C was stored in the 50-150 µm and macro-organic matter fractions of arable land with a sandy loam texture. According to Clemente et al. [36], the finest fractions (fine silt and clay) in a control calcareous soil had the highest concentrations of organic carbon but the coarsest (sand) fraction contained the highest

content (80% of the added carbon) of organic carbon in the same soil treated with humic acid of commercial peat. Accoe et al. [37] stated that in 0-10 cm and 10-20 cm layers of grassland soil, the largest C and N contents were found in the size fraction <50 µm.

The C/N ratio fluctuated around 10 and did not vary strongly between fractions, except the macro-organic matter fraction (Fig. 2). In the macro-organic matter fraction, C/N ratios exceeded 30 in unamended soil, compared to about 15 in the treated soil. Christensen [38] stated that the decrease of C-to-N ratios from the macro-organic matter fraction towards the <50 µm fraction is due to the increasing degree of humification. The transfer of C and N from the macro-organic matter fraction towards the 50-150 µm and <50 µm fractions is however a slow process and that the soil organic matter accumulation in these two fractions is only detectable after a long term [37].

3.4. Presence of trace metals in the different particle size-fractions of the soil

In the control soil, the total concentrations of Cu, Zn and Cr were higher in <50µm fraction than in the other fractions. The Cd concentration was higher in the macro-organic matter fraction. There were no significant differences in the concentrations of Pb and Ni between the different particle-size fractions. The addition of farmyard manure during seven successive years only increased significantly the Zn concentration in the macro-organic matter fraction (249 mg kg^{-1} fraction, compared to 109 mg kg^{-1} fraction in the control soil) and the Cu concentration in the 50-150 µm fraction (60.2 mg kg^{-1} fraction, compared to 38.5 mg kg^{-1} fraction in the control soil) (Table 2).

Application of municipal solid waste compost significantly increased the concentrations of Cu, Zn and Pb in the 50-150 µm, <50 µm and macro-organic matter fractions, and the

concentration of Cd in the 50-150 μm fraction. Municipal solid waste compost had no significant effect on the concentration of Ni and Cr in all size fractions.

Venditti et al. [39] also found that the highest metal concentrations are found in the finer particle-size fraction (<50 μm). They attributed this to the presence of clays, which have a high density of adsorption sites, in this fraction, as well as to the higher efficiency of acid digestion in this fraction, since the mineral components are more accessible to the reagents used during digestion. The clay minerals and the so-called humic-clay complexes are indeed mainly responsible for metal fixation in soils [40]. According to Qian et al. [41], trace metals accumulate in the clay (<2 μm) as well as the sand fraction (>125 μm). The accumulation of trace metals in the clay fraction is attributed to the high surface area and presence of clay minerals, organic matter, Fe-Mn oxides and sulphides. The higher metal concentration in the sand fraction results from the presence of heavy minerals which retain metals very strongly [41].

Maisonnave et al. [42] stated that the <20 μm fraction contains the highest concentrations of trace metals (mg kg^{-1} dry weight of soil), which was attributed to the presence of clay and Fe and Mn oxides. Some metals however tented to accumulate also in other fractions (>20 μm): Cd in the 20-50 μm fraction, Cu in the 50-100 μm fraction and Zn in the 100-500 μm fraction. Iwegbue et al. [43] stated that the fine fraction had the highest trace metal content and the coarse fraction contained the least amount of each trace metal. Next to clay and Fe and Mn oxides, organic matter also contributes to the retention of trace metals in soils by the formation of stable complexes with the active sites of the soil's organic ligands [44, 45]. Balabane and Van Oort, [46] stated that metal concentrations in particulate organic matter fractions were higher than that in the bulk soil samples and that they increased with decreasing particulate organic matter size. Besnard et al. [47] found heterogeneity in the

distribution of Cu among particle-size fractions of soil. The highest Cu contents were detected in particulate organic matter fractions. Thus, in non-amended and urban compost soil, the total particulate organic matter fraction was nearly 6 times richer in Cu than the bulk soil.

A real environmental hazard may exist at long-term when the trace metals would be released slowly over time as the added organic matter is decomposed [12]. Several authors claim that upon mineralization, the metals previously bound to the organic matter will indeed be remobilised into the environment. Others however claim that metals will be transferred from the more available fractions to e.g. highly insoluble organic complexes with strongly humified organic matter and thus sequestered in long-term sinks [29].

3.5. Chemical fractionation of trace metals in the soil

Figure 4 shows results of the application of the BCR sequential extraction scheme for the control, farmyard manure and municipal solid waste compost amended soil, together with results of the fourth, aqua regia extraction step, which determines the residual fraction. The recovery was calculated for all metals as the sum of all BCR fractions divided by the amount of metals released by aqua regia extraction, expressed as percentage. Recoveries for most of metals ranged from 80 % to 120 %. The recovery for Cd and Pb however ranged from 120 % to 130 %, which is explained by the very low concentrations of these metals.

Cadmium was mainly extracted in the first and second extraction step (acid-extractable and reducible fraction), both for the control and amended soils. The application of farmyard manure and municipal solid waste compost increased insignificantly the Cd concentration in the acid-extractable fraction. This concentration increased from 0.093 mg kg^{-1} in the control soil to 0.121 mg kg^{-1}, 0.122 mg kg^{-1} and 0.116 mg kg^{-1} after the application of 120 t ha^{-1} of farmyard manure, 80 t ha^{-1} and 120 t ha^{-1} of municipal solid waste compost, respectively.

Furthermore, the main increment was observed in the reducible fraction, which the concentration of Cd increased insignificantly from 0.100 mg kg^{-1} in control soil to 0.356 mg kg^{-1} after application of 120 t ha^{-1} of municipal solid waste compost. The two organic amendments had no effect on the Cd concentrations in the oxidisable and residual fraction. These results correspond well with those reported by Qiao et al. [48], who stated that the acid-extractable and reducible fraction retained a large proportion of Cd in clay soil amended with different rates of biosolids.

In the control soil, Cu seemed to be mainly present in the residual (60 %) and reducible fraction (30 %). Application of farmyard manure had no effect on the Cu distribution. Application of municipal solid waste compost however significantly increased the Cu concentration in all soil fractions, except the reducible fraction. Upon addition of 120 t ha^{-1} of municipal solid waste compost, the Cu concentration increased from 0.053 mg kg^{-1} in the acid extractable fraction of control soil to 0.558 mg kg^{-1} in amended soil and from 2.2 mg kg^{-1} in the oxidisable fraction of control soil to 13.3 mg kg^{-1} in amended soil. The latter indicates that Cu has a very high affinity to organic compounds, as also previously reported [49,51]. In the residual fraction, the Cu concentration increased from 25.4 mg kg^{-1} in the control soil to 51 mg kg^{-1} in amended soil. Qiao et al. [48] however stated that reducible Cu in red clay soil increased drastically with increasing biosolid application rates. Zheljazkov and Warman, [52] reported that application of municipal solid waste compost increased the Cu concentration in the oxidisable fraction, as well as exchangeable Cu concentrations. According to Kidd et al. [53], the concentration of Cu in the oxidisable fraction increased from 15% in a control soil to 53% after long-term application of sewage sludge to a sandy loamy soil.

The largest portion of Pb was found in the reducible fraction, both in the unamended and amended soils. Compared to the control soil, the farmyard manure had no effect on the distribution of Pb. Similar results were found by Parat et al. [31], who observed no differences

between control and manure-treated soil during 20 years at 10 t ha^{-1} y^{-1} for Cu, Zn and Pb concentrations. Municipal solid waste compost tended to increase the Pb concentration in all fractions, except the acid-extractable fraction. Zheljazkov and Warman [52] reported that municipal solid waste compost contributes to an important extent to the increment of the presence of Pb in the reducible fraction. Lead moreover forms strong complexes with organic matter in the soil. These complexes obtain a high degree of irreversibility, which hampers any future attempt to remediate Pb-polluted soils [54].

Zinc was principally distributed between the reducible and residual fractions. Farmyard manure increased insignificantly the Zn concentration in different fractions of soil. Municipal solid waste compost increased significantly the Zn concentrations in all soil fractions, proportional to the application rates. The highest increase was observed in the reducible and residual fractions. Indeed, the Zn concentration passed from 15.2 mg kg^{-1} in reducible fraction of unamended soil to 58.5 mg kg^{-1} and 96.5 mg kg^{-1} after the application of 80 t ha^{-1} and 120 t ha^{-1} of municipal solid waste compost, respectively. The long-term application of composted sewage sludge in a sandy loam soil increased the Zn bound to organic matter fraction from <5% to 44% [53]. Furthermore, the long-term application of municipal solid waste compost increased the concentration of Zn in acid-extractable, Fe and Mn oxides and organic matter fractions [52]. Qiao et al. [48] showed that the application of biosolids increased the Zn concentration in the reducible fraction of a Taihu paddy soil (Agri-Udic Ferrosol), and this may have been due in part of differences in soil pH. Planquart et al. [55] reported that the application of sewage sludge compost to three soils from France under greenhouse conditions increased the proportion of Zn bound to acid extractable fraction.

Nickel was largely present in the residual fraction in unamended and amended soil. These results are in accordance with those found by other authors [56]. While studying two untreated calcareous soils, Rajaie et al. [57] found that Ni was predominantly bound to oxides

and in the residual fraction (99.1% in clay loam and 98.7% in sandy loam respectively). The application of farmyard manure had no effects on the distribution of Ni in our soil. Municipal solid waste compost increased significantly the Ni concentration in reducible fraction, but only after the addition of 120 t ha^{-1}. The Ni concentration passed from 3.47 mg kg^{-1} in control soil to 5.06 mg kg^{-1} in amended soil. In the oxidisable fraction, the Ni concentration increased insignificantly after the application of both organic amendments. Contrary, Businelli et al. [58] found upon application of municipal waste compost to the superficial layer of a landfill-covering soil, the percentage of Ni in all fractions of the A horizon remained relatively constant. This is possibly due to the limited contribution of compost to the concentration of Ni in the A horizon.

Chromium was mainly distributed between the third and the fourth fraction: more than 95% of Cr in the soil was found in these fractions (Fig. 3). This indicates that under natural conditions Cr would not be easily released. When applying a modified BCR sequential extraction procedure, Zemberyov et al. [59] found that chromium was found exclusively in the residual fraction (47–65%) bound in mineral lattice. Application of municipal solid waste compost resulted in a significant increase of the Cr concentration in the reducible and oxidisable fraction. A pot experiment of Bose et al. [60] showed that upon addition of industrial waste, a maximum level of Cr was bound with Fe and Mn oxides. Due to the high affinity of Cr to organic matter, the solubility of this metal seemed to be limited by the formation of insoluble chromium-organic complexes [61]. Consequently, application of municipal waste compost during 10 years increased the Cr concentration in the organically-bound fraction of the superficial layer (A horizon) of a landfill-covering soil [58].

4. Conclusions

The application of Tunisian municipal solid waste compost instead of farmyard manure to a Tunisian calcareous soil during seven successive years significantly ameliorated the soil fertility under field conditions. Consequently, this amendment can be used to increase the organic matter content of Tunisian soil. However, the compost seems to be a source of accumulation of trace metals in the soil which could cause a real environmental hazard on the longer term. The distribution of trace metals between different particle-size fractions of soil showed the highest dominance of the macro-organic matter fraction in accumulating trace metals, both in untreated and in treated soil. The results of the sequential extraction indicate that all trace metals were initially mainly found in the residual fraction, except Cd and Pb in reducible fraction. Municipal solid waste compost and farmyard manure had no effects on the exchangeable metal fraction within the soil. Since a redistribution of trace metals in the treated soils may however occur upon decomposition of organic matter, especially the mobility and bioavailability of trace metals might increase on longer term and finally cause an environmental hazard. Longer-term field experiments are needed to completely assess the environmental risks of thee metals accumulating in the soils upon municipal solid waste compost application.

5. References

[1] F. Madrid, R. López, F. Cabrera, Metal accumulation in soil after application of municipal solid waste compost under intensive farming conditions. Agr Ecosyst Environ. 199 (2007) 249-256.

[2] N. Jedidi, Minéralisation et humification des amendements organiques dans un sol limono-argileux Tunisien. T.D. en Sciences Biologiques Appliquées. Université de Gand, Belgique, (1998) pp, 180.

[3] P. Bhattacharyya, K. Chakrabarti, A. Chakraborty, Residual effect of municipal solid waste compost on microbial biomass and activities in mustard growing soil. Archives of Agronomy and Soil Science 49 (2003) 585 – 592.

[4] A. N'Dayegamiye, A. Drapeau, R. Laverdière, Effets des apports de composts de résidus ménagers sur les rendements des cultures et certaines propriétés du sol. Agrsol. 16 (2005) 135-144.

[5] M. Basu, P. B. S. Bhadoria, S. S C. C. Mahapatra, Comparative effectiveness of different organic and industrial wastes on peanut: Plant growth, yield, oil content, protein content, mineral composition and hydration coefficient of kernels. Archives of Agronomy and Soil Science 53 (2007) 645 – 658.

[6] H. Cherif, F. Ayari, H. Ouzari, M. Marzorati, L. Brusetti, N. Jedidi, A. Hassen, D. Daffonchio, Effects of municipal solid waste compost, farmyard manure and chemical fertilizers on wheat growth, soil composition and soil bacterial characteristics under Tunisian arid climate. Eur J Soil Biol .(2009) doi:10.1016/j.ejsobi.2008.11.003.

[7] I. Celik, I. Ortas, S. Kilic, Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. Soil & Tillage Research 78 (2004) 59–67.

[8] C. P. Jordao, C. C. Nascentes, P. R. Cecon, R. L. Fontes, J. L. Pereira, Heavy metals availability in soil amended with composted urban solid wastes. Environ Monit Assess. 112 (2006) 309-326.

[9] J. C. Hargreaves, M. S. Adl, P. R. Warman, A review of the use of composted municipal solid waste in agriculture. Agric Ecosyst Environ. 123 (2008) 1–14.

[10] D. V. Pérez, S. Alcantra, C. C. Ribeiro, R. E. Pereira, G. C. Fontes, M. A. Wasserman, T. C. Venezuela, N. A. Meneguelli, C. A. A. Parradas Composted municipal waste effects on chemical properties of Brazilian soil. Biores Technol. 98 (2007) 525-533.

[11] S. R. Smith, A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environment International. 35 (2009) 142–156.

[12] M. B. Mc Bride, Toxicmetal accumulation from agricultural use of sludge: are USEPA regulations protective? J Environ Qual. 24 (1995) 5-8.

[13] A. Tessier, P. G. C. Compbell, M. Bison, Sequential extraction procedure for the speciation of particulate trace metals. Analyt Chem. 51 (1979) 844-850.

[14] G. Sposito, L. J. Lund, A. C. Chang, Trace metal chemistry in arid zone field soils amended with sewage sludge. I. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. Soil Sci Soc Am J. 46 (1982) 260-264.

[15] Shuman L M. Fractionation method for soil microelements. Soil Sci. 140 (1985) 11-22.

[16] G. Rauret, J. F. López-Sanchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, Ph. Quevauiller, Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J Environ Monit 1999; 1: 57-61.

[17] Sahuquillo A, Rigol A, Rauret G. Overview of the use of leaching/extraction tests of risk assessment of traces metals in contaminated soils and sediments. Trends Anal Chem. 22 (2003) 152-159.

[18] S. Cornu, B. Clozel, Extractions séquentielles et spéciation des éléments traces métalliques dans les sols naturels. Analyse critique. Etude et Gestion des Sols. 7 (2000) 179-189.

[19] G. Rauret, J. F. López-Sanchez, A. Sahuquillo, E. Barahona, M. Lachica, A. Ure, Application of a modified BCR sequential extraction (three step) procedure for the determination of extractable trace metal contents in sewage sludge amended soil reference

material (CRM483), complemented by a three-year study of acetic acid and EDTA extractable metal content. J Environ Monit. 2 (2000) 228-233.

[20] A, Walkley, I. A. Black, An examination degtijarf method for determination for role organic matter and proposed modification of the chromic acid titration method. Soil Sc. 37 (1934) 29-38.

[21] F. W. Meijboom, J. Hassink, K. M. Van Noordwij, Density fractionation of soil macro-organic matter using silica suspensions. Soil Biology and Biochemistry 27 (1995) 1109-1111.

[22] E. Van Ranst, M. Verloo, A. Demeyer, J. M. Pauwels, Manual for the soil chemistry and fertility laboratory. Analytical Methods for soils and Plants Equipment, and Management of Consumables (1999) pp, 243.

[23] Ph. Quevauviller Operationally defined extraction procedures for soil and sediment analysis. I. Standarization. Trends Anal Chem. 17 (1998) 289-298.

[24] W. Geebelen, D. C. Adriano, D. Van Der Lelie, M. Mench, R. Carleer, H. Clijsters, J. Vangronsveld, Selected bioavailability assays to test the efficacy of amendment – induced immobilization of lead in soils. Plant and Soil 249 (2003) 217-228.

[25] M. Soumare´, F. M. G. Tack, M. G. Verloo, Characterization of Malian and Belgian solid waste composts with respect to fertility and suitability for land application. Waste Management 23 (2003) 517–522.

[26] J. Weber, A. Karczewska, J. Drozd, M. Licznar, S. Licznar, E. Jamroz, A. Kocowic, Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. Soil Biol Biochem. 39 (2007) 1294-1302.

[27] O. Bouzaiane, H. Cherif, F. Ayari, N. Jedidi, A. Hassen, Municipal solid waste compost dose effects on soil microbial biomass determined by chloroform fumigation-extraction and DNA methods. Annals of Microbiology 57 (2007) 4: 1-7.

[28] M. V. Gil, M. T. Carballo, L. F. Calvo, Fertilization of maize with compost from cattle manure supplemented with additional mineral nutrients. Waste Management 28 (2008) 1432–1440.

[29] G. Du Laing, A. Bontinck, R. Samson, B. Vandecasteele, D. R. J. Vanthuyne, E. Meers, E. Lesage, F. M. G. Tack, M. G. Verloo, Effect of decomposing litter on the mobility and availability of metals in the soil of a recently created floodplain. Geoderma 147 (2008) 34-46.

[30] V. Illera, I. Walker, P. Souza, V. Cala, Short-term effects of biosolid and municipal solid waste application on heavy metals distribution in a degraded soil under a semi-arid environment. Sci Total Environ. 255 (2000) 29-44.

[31] C. Parat, R. Chaussodb, J. Lévequea, F. Andreux, Long-term effects of metal-containing farmyard manure and sewage sludge on soil organic matter in a fluvisol. Soil Biology & Biochemistry 37 (2005) 673–679

[32] R. Canet, F. Pomares, F. Tarazona, Chemical extractability and availability of heavy metals after seven years application of organic wastes to a citrus soil. Soil Use and Management 13 (1997) 117–121.

[33] G. Gigliotti, D. Businelli, P. L. Giusquiani Trace metals uptake and distribution in corn plants grown on a 6-year urban waste compost amended soil. Agricult Ecosyst Environ. 58 (1996) 199-206.

[34] I. Walter, F. Martınez, L. Alonso, J. Gracia, G. . Cuevas, Extractable soil heavy metals following the cessation of biosolids application to agricultural soil. Environmental Pollut. 117 (2002) 315–321.

[35] F. Accoe, P. Boeckx, O. Van Cleemput, G. Hofman, X. Hui, H. Bin, Ch. Guanxiong, Characterization of soil organic matter fractions from grassland and cultivated soils via C content and $\delta^{13}C$ signature. Rapid Commun Mass Spectrom. 16 (2002) 2157- 2164.

[36] R. Clemente, A. Escolar, M. P. Bernal, Fractionation of heavy metals in a contaminated soil amended with organic materials of different mineralisation rates. Bioresour. Technol. (2006) in press, doi:10.1016/j.biortech.2005.08.018.

[37] F. Accoe, P. Boeckx, J. Busschaert, G. Hofman, O. Van Cleemput, Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. Soil Biology & Biochemistry 36 (2004) 2075-2087.

[38] B. T. Christensen, Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science 20 (1992) 1-90.

[39] D. Venditti, S. Durécu, J. Berthelin, A multidisciplinary approach to assess history, environmental risks and remediation feasibility of soils contaminated by metallurgical activities. Part A: chemical and physical properties of metals and leaching ability. Arch Environ Con Tox. 38 (2000) 411-420.

[40] R. Clemente, M. P. Bernal, Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. Chemosphere 64 (2006) 1264-1273.

[41] J. Qian, X. Shan, Z. Wang, Q. Tu, Distribution and plant availability of heavy metals in different particle-size fractions of soils. The Science of the Total Environment 187 (1996) 131-141.

[42] V. Maisonnave, Ch. Bonnin, M. Vignoles, J. C. Revel, L. Patria, Impact des épandages de boues sur la teneur en éléments traces métalliques dans un sols et végétal. T S M. 4 (2003) 41-49.

[43] C. M. A. Iwegbue, F. N. Emuh, N. O. Isirimah, A. C. Egun, Fractionation, characterization and speciation of heavy metals in composts and compost-amended soils. African Journal of biotechnology. 6, 2(2007) 67-78.

[44] Y. Chen, Organic matter reactions involving micronutrients in soils and their effect on plants. In: Piccolo A. (Ed), Humic substances in terrestrial Ecosystems. Elsevier Amsterdam (1996) 507-580.

[45] A. T. G. Pigozzo, E. Lenzi, J. L. Junior, C. Scapin, A. C. S. Da Costa, Transition metal rates in latosol twice treated with sewage sludge. Brazilian archives of biology and technology 49, 3 (2006) 515-526.

[46] M. Balabane, F. Van Oort, Metal enrichment of particulate organic matter in arable soils with low metal contamination. Soil Biology & Biochemistry 34 (2002) 1513–1516.

[47] E. Besnard, C. Chenu, M. Robert, Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soils. Environmental Pollution 112 (2001) 329-337.

[48] X. L. Qiao, Y. M. Luo, E. P. Christi, M. H. Wong, Chemical speciation and extractability of Zn, Cu an Cd in two contrasting biosolids-amended clay soils. Chemosphere 50 (2003) 823-829.

[49] G. Du Laing, E. Meers, M. Dewispelaere, J. Rinklebe, B. Vandecasteele, M. G. Verloo, F. M. G. Tack, Effect of water table level on metal mobility at different depths in wetland soils of the Scheldt estuary (Belgium). Water Air Soil Pollut. (2009) in press, doi: 10.1007/s11270-009-9982-2.

[50] M. C. M. A. Ferraz, J. C. N. Lourenco, The influence of organic matter content of contaminated soils on the leaching rate of heavy metals. Environ Prog. 19 (2000) 53–8.

[51] K. Kalbitz, R. Wennrich, Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. Sci Total Environ. 209 (1998) 27–39.

[52] V. Zheljazkov, P. R. Warman, Source separated municipal solid waste compost application to Swiss chard and basil. J Environ Qual. 33 (2004) 542-552.

[53] P. S. Kidd, M. J. Dominguez-Rodriguez, J. Diez, C. Monterroso, Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere 66 (2007) 1458-1467.

[54] H. M. Poggi-Varaldo, N. Rinderknecht-Seijas, A differential availability factor for the evaluation of pollutant availability in soil treatments. Acta Biotechnol. 23 (2003) 271-280.

[55] P. Planquart, G. Bonin, A. Prone, C. Massiani, Distribution, movement and plant availability of trace metals in soils amended with sewage sludge composts: application to low metal loadings. The Science of the Total Environment 241 (1999) 161-179.

[56] Narwal R P, Singh B R. effects of organic materials on portioning, extractabibilty and plant uptake of metals in alum shale soil. Water Air and Soil Pollut. 203 (1998) 405-421.

[57] M. Rajaie, N. Karimian, J. Yasrebi, Nickel transformation in two calcareous soil textural classes as affected by applied nickel sulfate. Geoderma 144 (2008) 344–351.

[58] D. Businelli, L. Massaccesi, D. Said-Pullicino, G. Gigliotti, Long-term distribution, mobility and plant availability of compost-derived heavy metals in a landfill covering soil. Since of the Total Environment (2009) 1426-1435.

[59] M. Zemberyov, J. Bartekov, I. Hagarov, The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. Talanta. 70 (2006) 973–978.

[60] S. Bose, A. Jain, V. Rai, A. L. Ramanathan, Chemical fractionation and translocation of heavy metals in Canna indica L. grown on industrial waste amended soil. Journal of Hazardous Materials 160 (2008) 187–193.

[61] P. Bhattacharyya, A. Chakraborty, K. Chakrabarti, S. Tripathy, M. A. Powell, Chromium uptake by rice and accumulation in soil amended with municipal solid waste compost. Chemosphere 60 (2005) 1481-1486.

[62] J. Schoeneau, Impacts of repeated manure additions on soil fertility. In: Proceedings of the Soils and Crops. University of Saskatchewan Extension Press, Saskatoon, S.K., (On. CD.), (2005) 95-100.

[63] P.Qian, J.J.Schoenau, T.Wu, S.P. Mooleki, Copper and zinc amounts and distribution in soils as influenced by application of animal manure in east-central Saskatchewan. Can. J. Soil Sci. 83(2003) 197-202.

Table 1. Characteristics of soil, manure and compost used in the experiment (mean ± Standard deviation, n =3). Results are expressed on dry weight basis.

	Soil	Manure	Compost
pH (H_2O)	8.4 ± 0.2	7.4 ± 0.6	7.2 ± 0.3
EC (mS/cm)	0.16 ± 0.03	2.5 ± 0.2	6.5 ± 0.7
CEC (meq/100g)	17.9 ± 2.0	24.6 ± 3.7	33.1 ± 4.1
C (g/kg)	10 ± 2	362 ± 32	202 ± 21
N (g/kg)	0.9 ± 0.1	13.0 ± 1.0	10.6 ± 2.0
C/N	11.4	27.8	19.1
OM (g/kg)	17.5	724	404
HR (%)	8.2	71.6	25.8
Clay (%)	29.1	-	-
Silt (%)	51.9	-	-
Sand (%)	18.4	-	-
Texture	Loamy - Clayey	-	-
Trace metals (mg/kg)			
Cu	50 ± 7	26 ± 3	278 ± 22
Zn	86 ± 8	120 ± 18	410 ± 26
Pb	47 ± 6	10 ± 1	325 ± 24
Cd	1.0 ± 0.2	0.7 ± 0.2	3.3 ± 0.4
Ni	31 ± 7	22 ± 4	44 ± 7
Cr	54 ± 6	24 ± 3	52 ± 9

HR: relative humidity

Table 2. Soil organic carbon, nitrogen and trace metals contents in the 0-20 cm soil layer after a 7-year application of manure (M) and compost (C) at different rates (40, 80 and 120 t ha^{-1}) (mean ± Standard deviation, n=4). Results are expressed on soil dry weight basis.

	T	M 40	M 120	C 40	C 80	C 120
C (g kg-1)	11 ±0.3 ^{a}	21±0.6 ^{b}	33±0.5 ^{c}	21±0.6 ^{b}	29±0.4 ^{ab}	35 ±0.3 ^{c}
N (g kg^{-1})	1.1 ±0.01 ^{a}	1.7 ± 0.04 ^{b}	2.8±0.03 ^{c}	1.5±0.04 ^{ab}	1.8 ±0.02 ^{b}	2.4± 0.04 ^{c}
Cu (mg kg^{-1})	42.8± 2.7 ^{a}	43.3 ± 7.0 ^{a}	50.5±2.0 ^{ab}	60.3±6.3 ^{bc}	67.0±3.9 ^{cd}	82.13±18.8 ^{d}
Zn (mg kg^{-1})	88 ± 4 ^{a}	85±2 ^{a}	117±24 ^{ab}	121± 11 ^{ab}	135± 12 ^{b}	212±41 ^{c}
Pb (mg kg^{-1})	27.1 ±2.3 ^{a}	28.7±6.2 ^{ab}	29.8± 1.4 ^{ab}	38.3±5.3 ^{ab}	50.0±8.2 ^{b}	83.5±25.6 ^{c}
Cd (mg kg^{-1})	0.28 ±0.03 ^{a}	0.27 ±0.08 ^{a}	0.32±0.01 ^{a}	0.38±0.04 ^{a}	0.40±0.03 ^{a}	0.64±0.29 ^{b}
Ni (mg kg^{-1})	20.9±0.3 ^{c}	20.1 ±0.4 ^{b}	19.6±0.6 ^{a}	22.0±0.4 ^{d}	21.4±0.6 ^{cd}	21.3±0.5 ^{cd}
Cr (mg kg^{-1})	51.5 ±1.7 ^{c}	45.4±1.6 ^{a}	48.3±3.3 ^{ab}	54.3±1.6 ^{c}	50.1 ±1.4 ^{bc}	50.3± 3.1 ^{bc}

Values at the same line followed by the same letter are not significantly different at $p<0.05$.

M and C: rates (t ha^{-1}) of manure and compost, respectively. T: untreated soil.

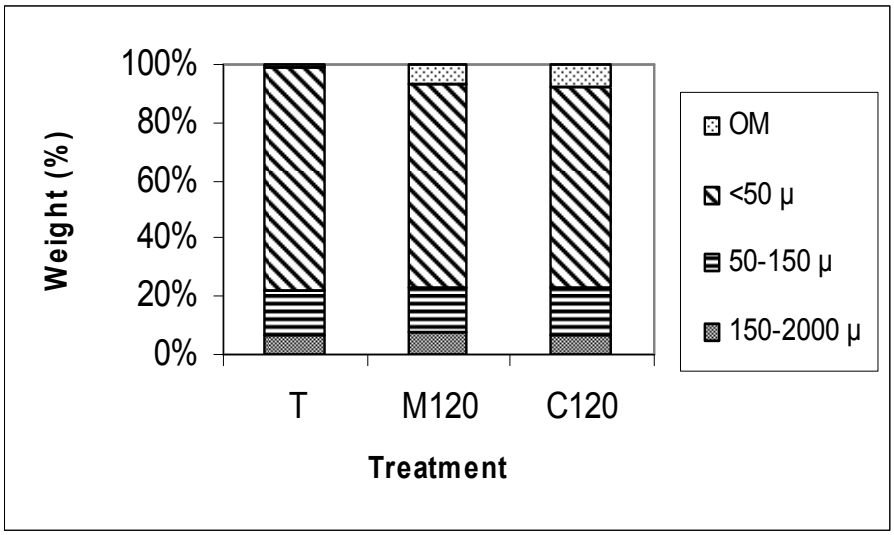

Fig. 1. Relative abundance of the different particle-size fractions within the soil (weight percentage).

T: unamended soil, F120: 120 t/ha of manure, C120: 120 t/ha of compost and OM: organic matter.

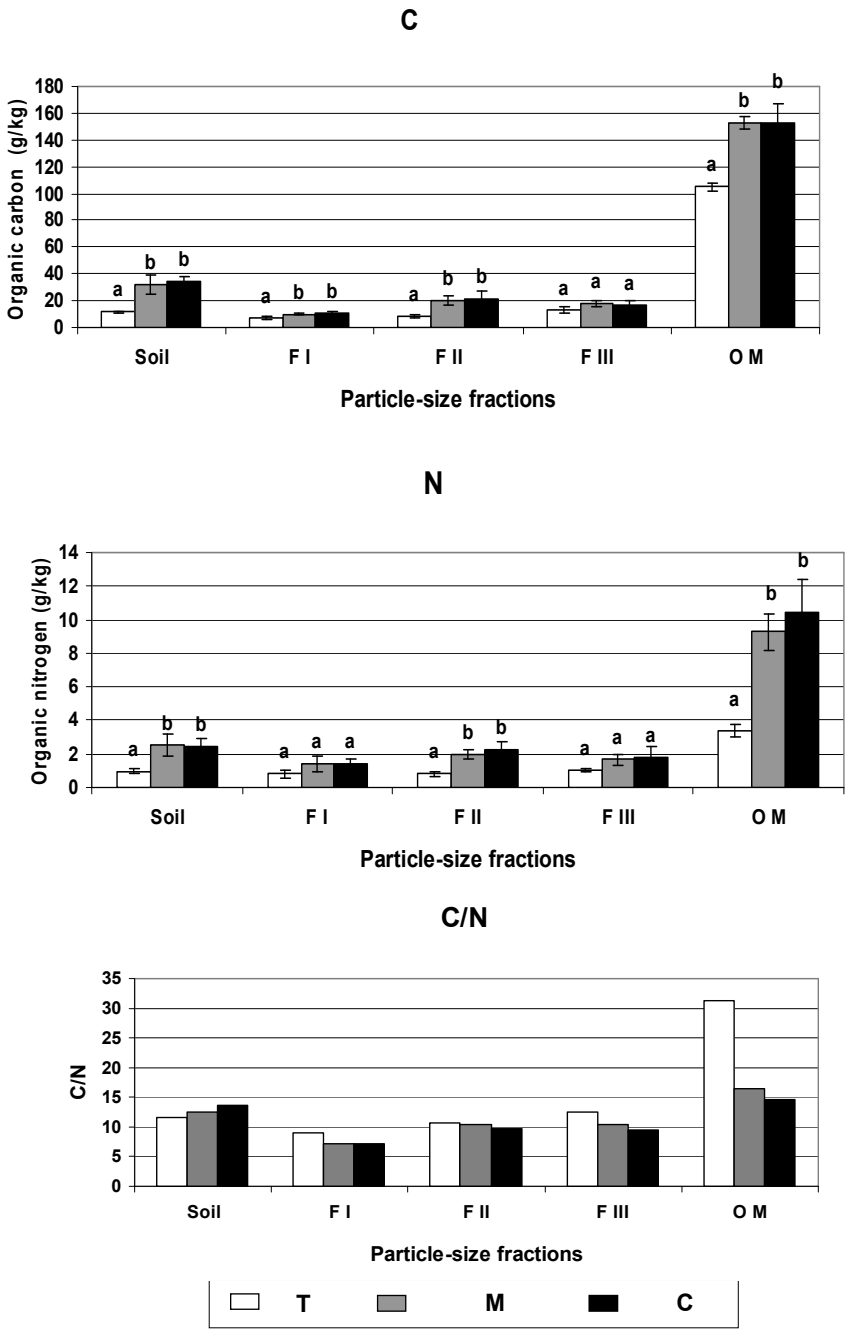

Fig. 2. Organic carbon (C) and nitrogen (N) concentrations and carbon/nitrogen ratio (C/N) in the different particle-size fractions of the soil (g kg^{-1} fraction).

Bars showing the same letter at the same soil fraction were not significantly different, according to Duncan's test at 5% probability level (n = 3). T: unamended soil, M: 120 t/ha of manure, C: 120 t/ha of compost. FI: >150 µm, FII: 150-50 µm and FIII: <50 µm fractions; OM: macro-organic matter fraction. (Mean ± Standard deviation, n=4).

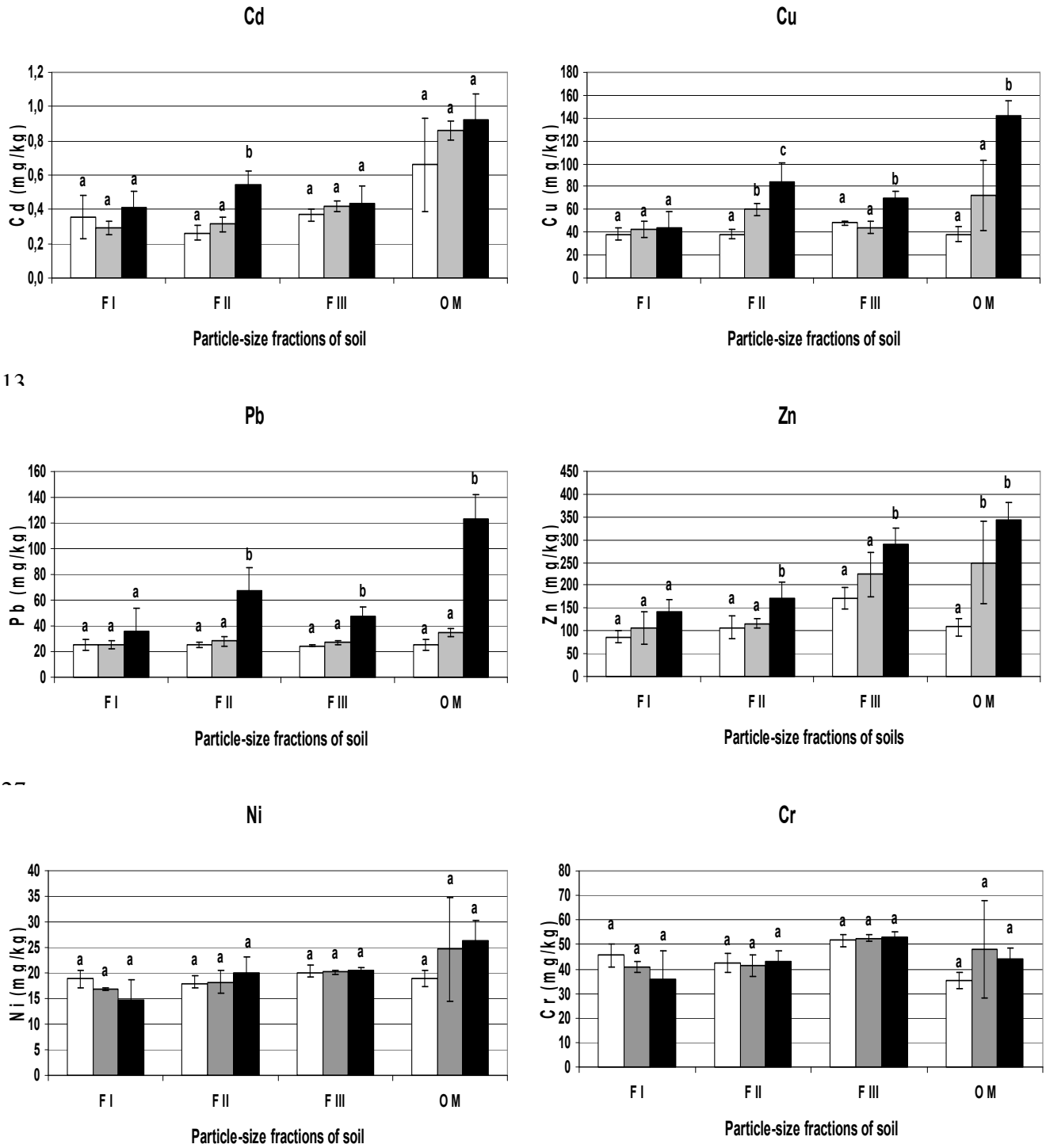

Fig.3. Cd, Cu, Pb, Zn, Ni and Cr contents in the different particle-size fractions of the soil (mg kg^{-1} fraction).

Bars showing the same letter at the same soil fraction were not significantly different, according to Duncan's test at 5% probability level (n = 4). T: unamended soil, M: 120 t/ha of manure, C: 120 t/ha of compost. FI: >150 µm, FII: 150-50 µm and FIII: <50 µm fractions; OM: macro-organic matter fraction. (Mean ± Standard deviation, n=4).

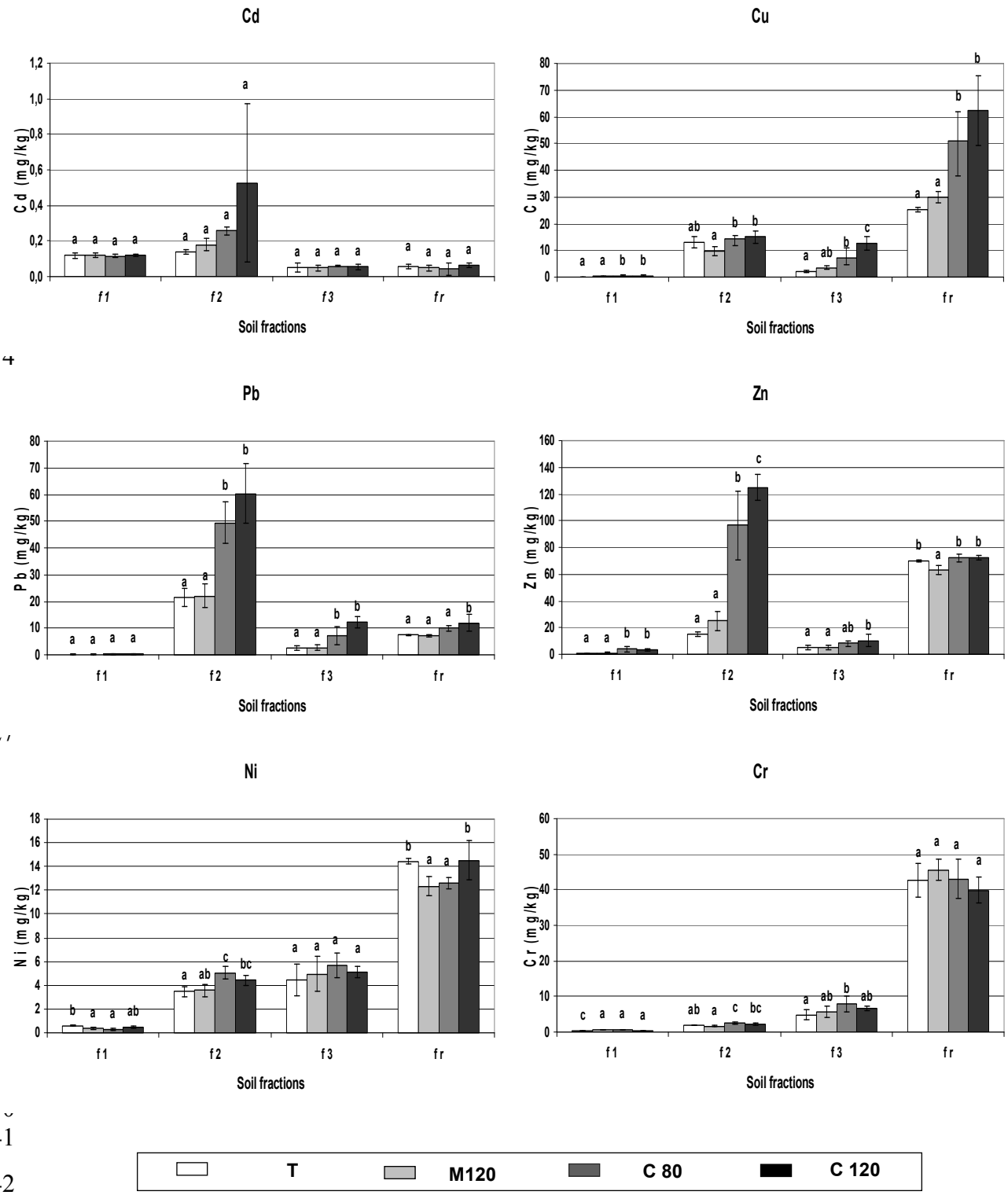

Fig.4. Fractionation of trace metals in the soil according to the BCR sequential extraction procedure.

Bars showing the same letter at the same soil fraction were not significantly different, according to Duncan's test at 5% probability level (n = 4). T: unamended soil, M120: 120 t/ha of manure, C80 and C120: 80 and 120 t/ha of compost. f_1: Acid extractable fraction, f_2: Reducible fraction, f_3: Oxidisable fraction and fr: Residual fraction. (Mean ± Standard deviation, N=4). Results are expressed on soil dry weight basis.